α-Actinin: Immunofluorescent Localization of a Muscle Structural Protein in Nonmuscle Cells

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Summary

Antibodies specific for the skeletal muscle structural protein α-actinin are used to localize this protein by indirect immunofluorescence in nonmuscle cells. In cultured nonmuscle cells, α-actinin is localized along or between actin filament bundles producing an almost regular periodicity. The protein is also detected in the form of fluorescent plaques at some ends of actin filament bundles, as well as in a filamentous form in some overlap areas of cells. In spreading rat embryo cells, α-actinin assumes a focal distribution which corresponds to the vertices of a highly regular actin filament network. The results suggest that α-actinin may be involved in the organization of actin filament bundles, in the attachment of actin filaments to the plasma membrane, and in the assembly of actin filaments in areas of cell to cell contact.

Introduction

Actin is a major structural component of many eucaryotic cells (Pollard and Weihing, 1974). In fully spread out tissue culture cells, actin filaments are organized into bundles which frequently span the whole length of the cell. Electron microscopic studies have indicated that some of these bundles may terminate at the plasma membrane in regions where the cell attaches to the underlying substrate or where two cells make contact (Abercrombie, Heaysman, and Pegrum, 1971; Heaysman and Pegrum, 1973). In a skeletal muscle sarcomere, actin filaments attach to the "Z-line" and emerge from it on either side with opposite polarities.

α-Actinin is a rod-shaped molecule with an approximate molecular weight of 95,000 daltons, a length of 300Å, and a diameter of 20Å. This protein has been localized exclusively in the Z-line (Stromer and Goll, 1972; Schollmeyer et al., 1974a), and in vitro studies have shown that α-actinin binds to actin filaments and forms regular cross-links between filaments, thereby generating parallel arrays (Podlubnaya et al., 1975). Such studies have indicated a probable role for α-actinin in the organization of actin filaments within the muscle sarcomere. In contrast to the structural organization of actin filaments in muscle, in nonmuscle cells little is currently known about the assembly of actin filament bundles or their interaction with the plasma membrane.

We have recently initiated immunofluorescence studies with antibodies to skeletal muscle α-actinin to determine the distribution of α-actinin in nonmuscle cells. In this paper we report that a protein which cross-reacts with antibodies against skeletal muscle α-actinin is localized at periodic intervals both within and between the actin filament bundles of nonmuscle cells, in regions of cell overlap, and at the ends of actin filament bundles. The results indicate that α-actinin may be intimately involved in the organization of the actin filament bundles of nonmuscle cells and may be important in their attachment to cell membranes.

Results

Characterization of the α-Actinin Antibody

We have previously demonstrated that the antibodies used in this study are highly specific for skeletal muscle α-actinin, as judged both by immunodiffusion and immunoelectrophoresis (E. Lazarides, manuscript in preparation). Since α-actinin has been shown to be exclusively localized in the Z-lines of muscle sarcomeres (Stromer and Goll, 1972; Schollmeyer et al., 1974a), we have further tested the specificity of our antibody preparation by its pattern of staining of skeletal myofibrils. The results are illustrated in Figures 1 and 2, which show a myofibril stained with the α-actinin antibody and examined first by phase contrast optics and then by epifluorescent optics. Only the Z-lines show fluorescence. Compared to unstained myofibrils, the Z-lines appear particularly dense in Figure 1, indicating the high levels of antibody binding in this structure.

In our preliminary work, we have isolated a protein which is similar to skeletal muscle α-actinin from a variety of nonmuscle sources (embryonic chick brain, cultured chick embryo fibroblasts, HeLa cells, mouse 3T3 cells, and human blood platelets). This protein can be extracted from these cells under the low ionic strength conditions used to extract α-actinin from muscle Z-lines (Goll et al., 1972), can be partially purified by chromatography on diethylaminoethyl (DEAE) cellulose, and is shown in centrifugation studies to bind to F-actin in 0.15 M KCl. In sodium dodecyl sulfate polyacrylamide gel electrophoresis, this partially purified component comigrates with skeletal muscle α-actinin,

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although on some gels the putative nonmuscle α-actinin can be resolved as a doublet (unpublished results). These results are in accordance with those of Schollmeyer et al. (1974b), who reported that a molecule with biochemical and immunological properties similar to those of muscle α-actinin could be identified in extracts of fibroblasts, the acrosomal filaments of Limulus sperm, and the microvilli of intestinal epithelial cells.

Since still very limited amounts of this protein can be obtained from nonmuscle sources, we have prepared antibodies against skeletal muscle α-actinin to examine the distribution of this protein and its interaction with other structural proteins in nonmuscle cells by indirect immunofluorescence. The cells used in this work are primary rat embryo cells, but similar results have been obtained with the established cell lines 3T3 and balb 3T3, as well as with primary human skin fibroblasts.

Immunofluorescent Localization of α-Actinin in Tissue Culture Cells

Fully spread out cells grown in tissue culture are known to contain fibers visible with phase contrast optics. These fibers frequently span the whole length of the cell, and they have been shown to correspond to the microfilament bundles observed with electron microscopy (Buckley and Porter, 1967). Binding of heavy meromyosin (Ishikawa, Bischoff, and Holtzer, 1969) and indirect immunofluorescence studies with antibodies specific for actin (Lazarides, 1975a) have demonstrated the actin composition of phase contrast fibers. Using antibodies specific for tropomyosin, it was further shown by indirect immunofluorescence that tropomyosin is also localized within these fibers (Lazarides, 1975a, 1975b). These observations led to the suggestion that the phase contrast fibers are aggregates of actin filaments with tropomyosin bound to them. Using the phase contrast fibers as a frame of reference, Figures 3 and 4 show that α-actinin is also localized within these filament bundles. In addition, however, this molecule is also found localized between the phase contrast fibers.

Analysis of the "Periodicity" Observed with the α-Actinin Antibody

We have previously shown that even at high magnification the actin antibody exhibited a continuous fluorescence along these filament bundles. The tropomyosin antibody, however, produced a periodic fluorescence which was well resolved in some fibers when viewed under oil immersion (100X) (Lazarides, 1975a, 1975b). Similarly, Figures 5 and 6 demonstrate that the α-actinin antibody also produces a periodic fluorescence which is clearly resolved along most cytoplasmic fibers. The lengths of the fluorescent segments and of the nonfluorescent segments along the filament bundles are both variable. Approximate values are 0.4 μm for the fluorescent segments, and 1.2 μm for the nonfluorescent intervals. As previously shown, the fluorescent segments seen with the tropomyosin antibody have an average length of approximately 1.2 μm, while the nonfluorescent segments are approximately 0.4 μm long (Lazarides, 1975b). When the α-actinin and the tropomyosin antibodies are applied sequentially to cells in appropriate dilutions, they produce a staining pattern without any striations, which is closely similar to that seen with the actin antibody (see Figure 12). These results suggest that the two types of striations seen with the α-actinin and the tropomyosin antibody are complementary.

In addition to the periodic segments, Figure 6 shows an example of the frequently enhanced fluorescence of the α-actinin antibody at regions where many filament bundles terminate. Figure 11 further illustrates that α-actinin can be found in regions of cell overlaps where groups of filament bundles may also be seen to terminate.

Transformations in the Distribution of α-Actinin

Apart from the periodic segments described above, in some cells α-actinin assumes a focal distribution. Such fluorescent foci are most pronounced in the initial stages of spreading of freshly plated cells (Figures 8 and 10). At later stages of spreading, the foci become progressively diminished as the striated pattern normally seen in the fully spread out cells begins to reappear. The foci in spreading cells correspond to the vertices of a complex but highly regular network of actin filaments that can be detected by phase contrast optics and actin antibody staining (Figures 7 and 9) (E. Lazarides, manuscript in preparation). The existence of these foci in both spreading and in certain, but not all, fully spread out cells indicates that their formation may be cell cycle- or cell shape-dependent.

Discussion

The results presented above demonstrate that antibodies to skeletal muscle α-actinin can be used to localize this protein in nonmuscle cells. The periodic arrangement of α-actinin along the actin filament bundles of nonmuscle cells is reminiscent of the highly ordered arrangement of this protein in skeletal myofibrils (compare Figures 2, and 5 and 6). Unlike the arrangement of this protein in myofibrils, however, the length of the periodic segments revealed with the α-actinin antibody is variable in nonmuscle cells. Particularly in some of the most prominent bundles (observed with phase contrast

optics), the fluorescence may even be continuous. This variable spacing of α-actinin along the actin filament bundles may result from a poor alignment of the constituent actin filaments within the bundles, possibly due to the locomotory activity of the cell. The actin filaments within these filament bundles would therefore seem to possess a greater lateral freedom of movement than the actin filaments in skeletal muscle sarcomeres, where the ordered register of the filaments is strictly preserved throughout contraction.

Dense regions within the actin filament bundles (microfilament bundles) of tissue culture cells, possibly representing homologous structures to muscle Z-lines, have been observed by electron microscopy (Buckley and Porter, 1967; Wessells, Spooner, and Ludueña, 1973; Goldman et al., 1975). The variable periodic distribution of α-actinin along the length of the actin filament bundles illustrated above is consistent with the observations of Wessells et al. (1973), who have commented that the distance between these dense bodies is also variable (approximately 0.6–1.2 μm). The role of these presumed α-actinin-containing dense bodies is uncertain. If, however, they are homologous to muscle Z-lines, and since α-actinin has been shown in vitro to cross-link and organize actin filaments (Podlubnaya et al., 1975), then it seems probable that α-actinin may also serve these functions in the filament bundles of nonmuscle cells.

Besides the fluorescent periodic segments found within the actin filament bundles, Figure 6 shows that the α-actinin antibody reveals also scattered fluorescent "patches" (marked p). These patches are usually found in regions where groups of filaments terminate or converge to "focal points." These areas may represent attachment plaques—the dense structures observed in cultured fibroblasts by electron microscopy, where the cells adhere to the underlying substratum and at which regions the actin filament bundles terminate (Abercrombie et al., 1971; Heaysman and Pegrum, 1973; Goldman et al., 1975).

Areas of cell to cell contact or overlap frequently show fluorescence with the α-actinin antibody, usually in a filamentous form (see Figure 11). These regions of bright fluorescence give the appearance of a continuity between the two adjacent cells, and they may represent areas of close membrane contact between the two cells. These areas may be identical to those described at the electron microscopic level by Heaysman and Pegrum (1973), who found that regions of close contact with increased electron dense material on each side may develop between adjacent cell membranes when two tissue culture cells collide. These transient structures appear to be similar to regions of cell substrate adhesion, and again filaments may be seen emerging from the regions of electron density (Heaysman and Pegrum, 1973). The presence of the α-actinin fluorescence within such areas suggests that this molecule may be intimately involved in the organization of these structures and in the attachment of actin filaments to the membrane in these regions. Actin filaments have been shown to interact with cell membranes in a variety of cell types: by electron microscopy (Pollard and Korn, 1973), biochemically (Spudich, 1974; Gruenstein, Rich, and Weihing, 1975; Murray and Dubin, 1975), and in reconstitution experiments with cytoplasmic membranes and filamentous (F) actin (Burridge and Phillips, 1975). Moreover, the similarity and possible homology of the muscle Z-line with particular specialized membrane junctions between cells (desmosomes) was suggested as early as 1964 by Franzini-Armstrong and Porter (1964), and a continuity between Z-lines and desmosomes has been demonstrated for certain types of muscle (Heuson-Stienon, 1965; Warren and Porter, 1969; Hagopian and Spiro, 1970). As described elsewhere (F. Miller, J. Elias, and E. Lazarides, manuscript in preparation), the α-actinin antibody used for the work presented here has been applied to frozen tissue sections, and it was found to stain, in addition to the Z-lines of the myofibrils, the intercalating discs and desmosomes of heart muscle. Similar results with regard to the localization of α-actinin in desmosomes have been presented by Schollmeyer et al. (1974b). There is therefore an increasing body of evidence to support the belief that α-actinin participates in the attachment of actin filaments to membranes.

We have previously shown that during cell spreading, rat embryo cells develop transiently a highly regular network of actin filaments, the vertices of which contain α-actinin, while the connecting actin fibers contain tropomyosin (E. Lazarides, manuscript in preparation). Examples of this focal localization of α-actinin are shown in Figures 8 and 10. This transient distribution of α-actinin foci precedes the periodic arrangement of this protein within the actin filament bundles of fully spread out cells, but it is also commonly observed in a minority of fully spread out cells. These observations suggest that the focal arrangement of α-actinin may be cell cycle- or cell shape-dependent, and demonstrate that α-actinin may change its distribution with corresponding changes in the state of aggregation or distribution of the cytoplasmic actin filaments.

Experimental Procedures

Cell Culture

Primary cultures of rat embryo cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. The cells were used between the second and third month of subcultivation.

For indirect immunofluorescence, cells were trypsinized at confluency for 5 min with 0.05% trypsin, 0.5 mM ethylene diamine tetraacetate (EDTA) in phosphate buffered saline (PBS), and seeded on 12 mm round glass coverslips.

Indirect Immunofluorescence and Antibody Preparation to α-Actinin

The antibodies were produced in white female New Zealand rabbits. Care was taken to keep the rabbits in separate rooms from other animals such as mice or hamsters. Antibodies produced in rabbits that were kept in rooms together with mice gave a very strong nuclear fluorescence in indirect immunofluorescence. This nuclear fluorescence was found to be minimal in the antibody preparation made in rabbits that were kept separate from other animals. The details of the antibody preparation to purified porcine skeletal muscle α-actinin, the immunological characterization of this antibody preparation, as well as the indirect immunofluorescence technique used for the localization of the α-actinin antibody, have been presented elsewhere (E. Lazarides, manuscript in preparation). The cells were viewed with epifluorescent optics in a Zeiss PMII microscope using a 63X oil immersion lens (100X in Figures 1 and 2).

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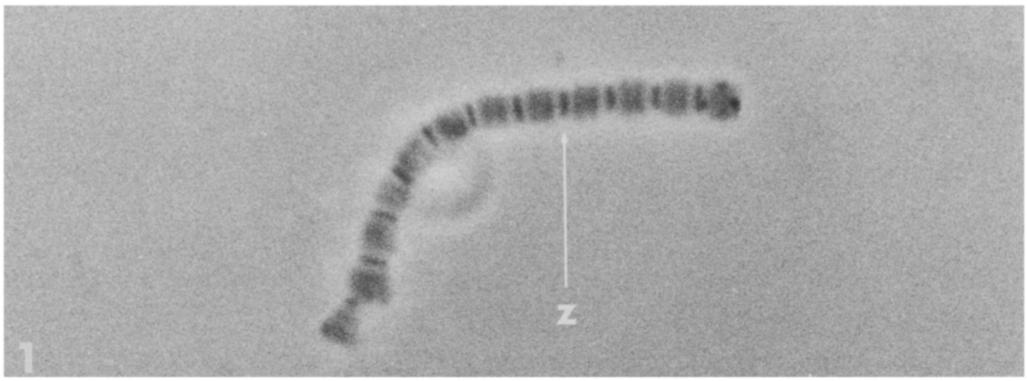
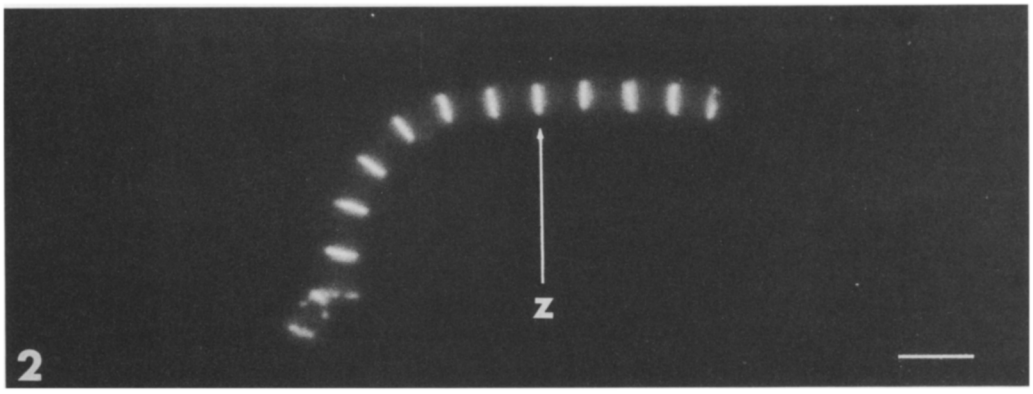
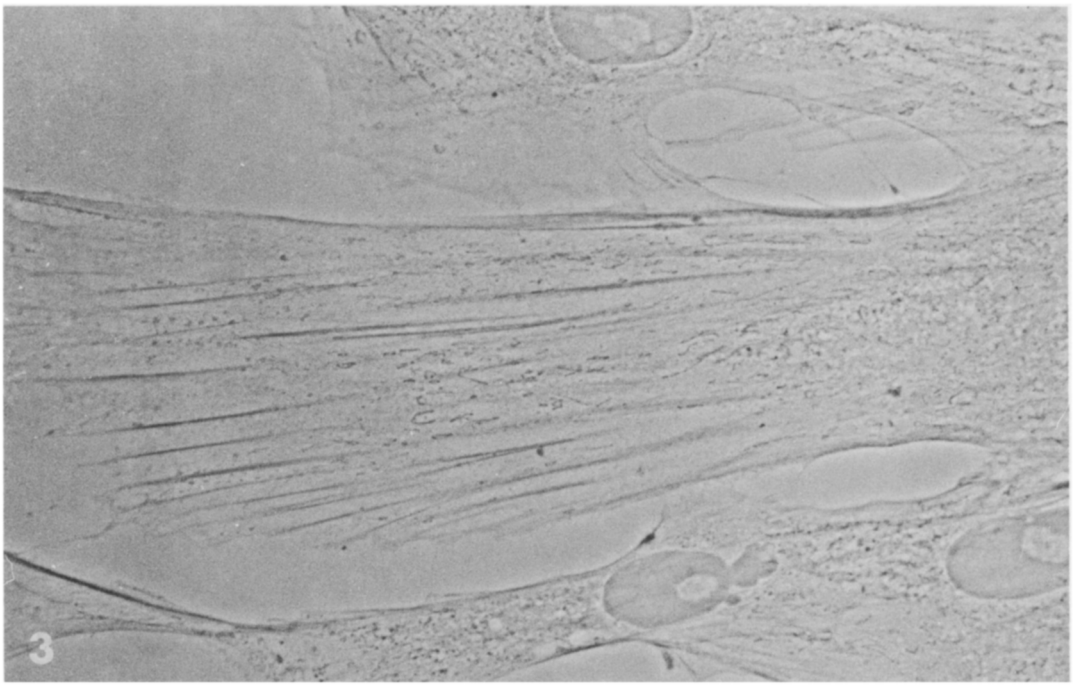
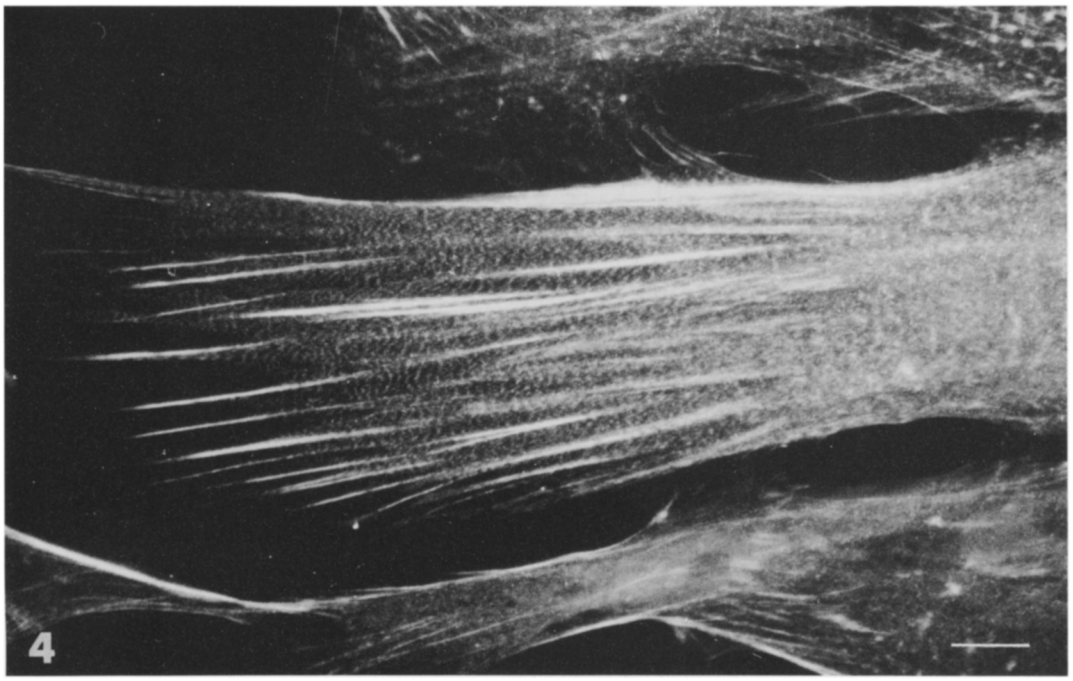




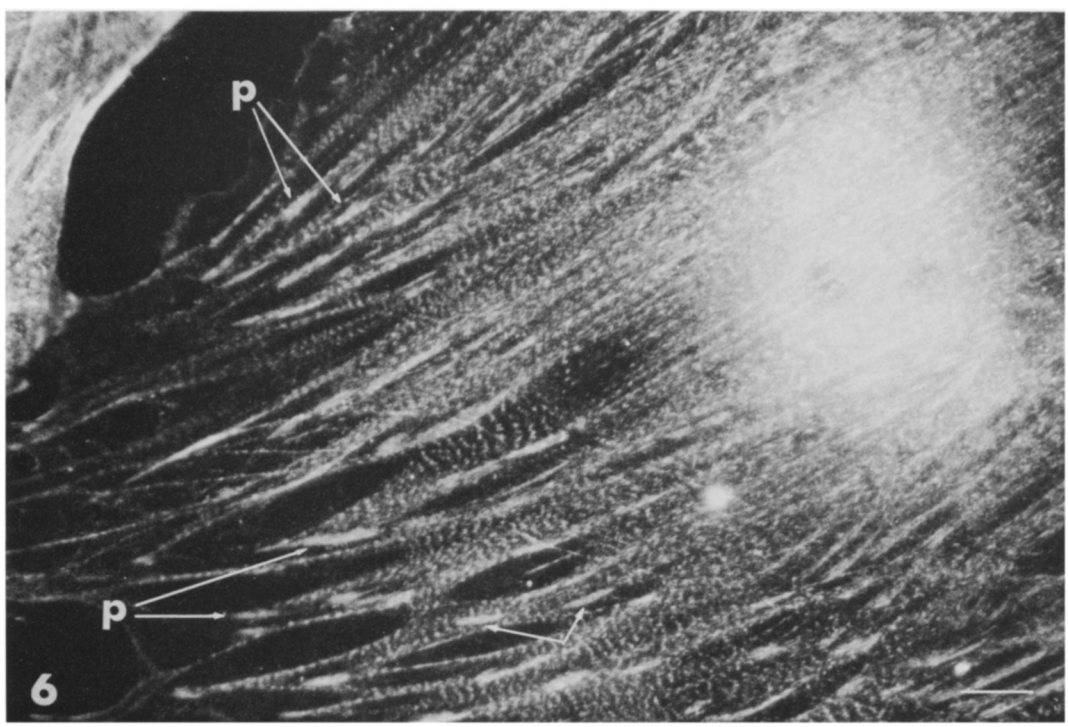
Figures 1 and 2. Indirect Immunofluorescence with the α-Actinin Antibody on Rat Skeletal Myofibrils

Myofibrils were stained with the antibody as described in Experimental Procedures and viewed with phase contrast (Figure 1) and epifluorescent (Figure 2) optics. The arrow termed Z refers to the localization of the Z-line within a sarcomere. There is an increased phase contrast of the Z-line in Figure 1 as a result of the antibody reaction with this structure. The distance between two Z-lines is approximately 2.6 μm. Bar = 5 μm.



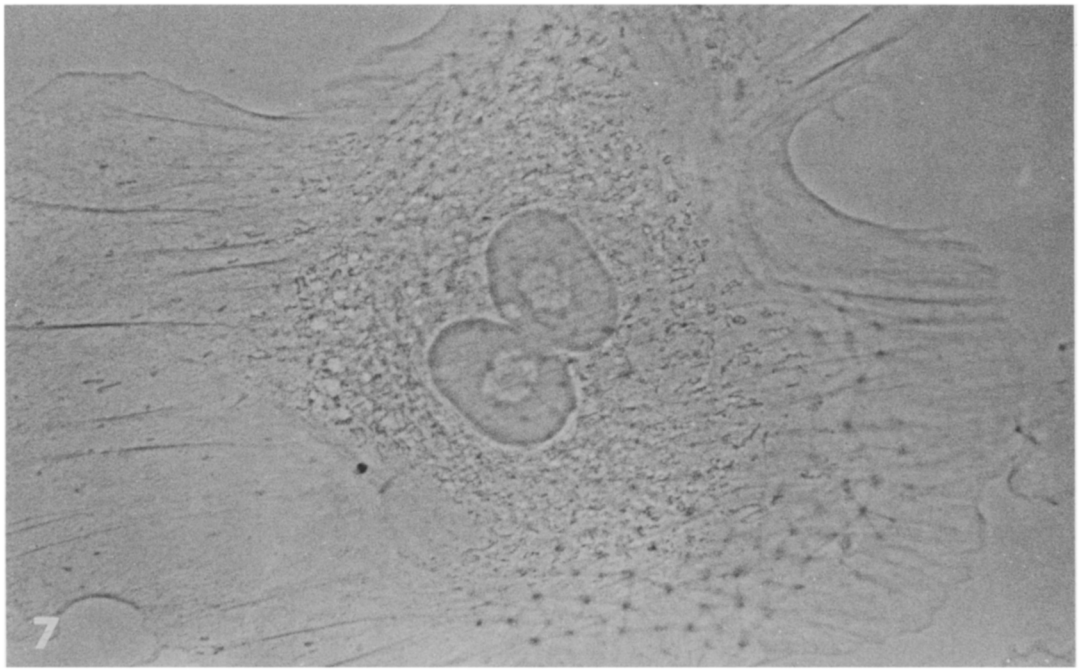


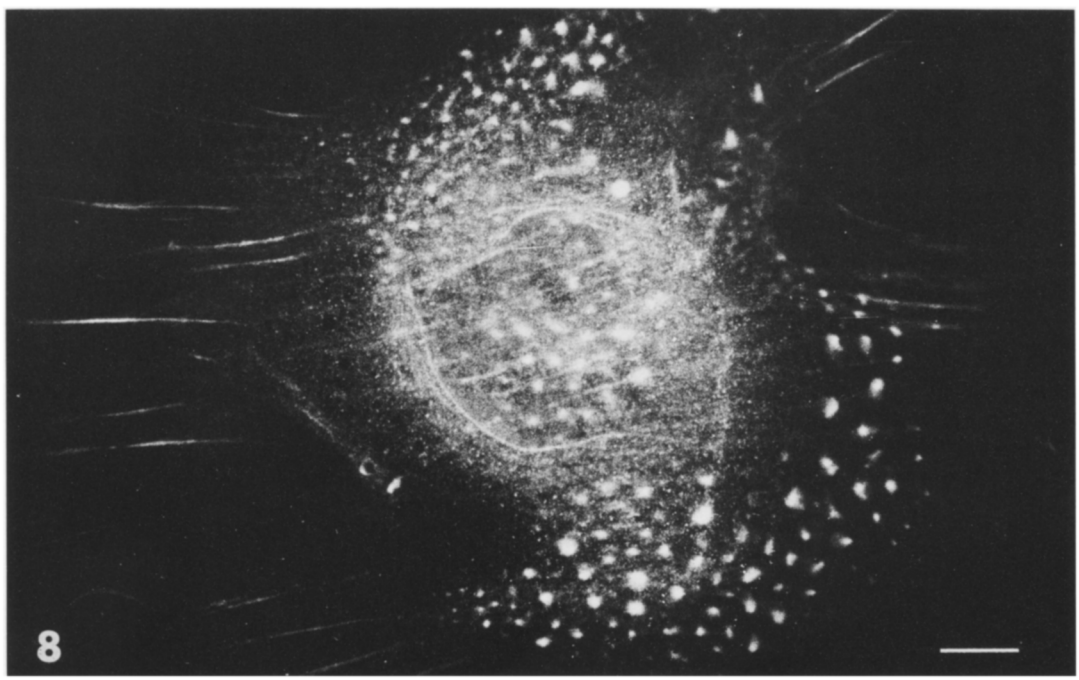
Figures 3 and 4. Indirect Immunofluorescence with the α-Actinin Antibody on a Fully Spread Out Rat Embryo Cell
The cells were reacted with the antibody and viewed with phase contrast (Figure 3) and epifluorescent (Figure 4) optics. Bar = 10 μm.



Figures 5 and 6

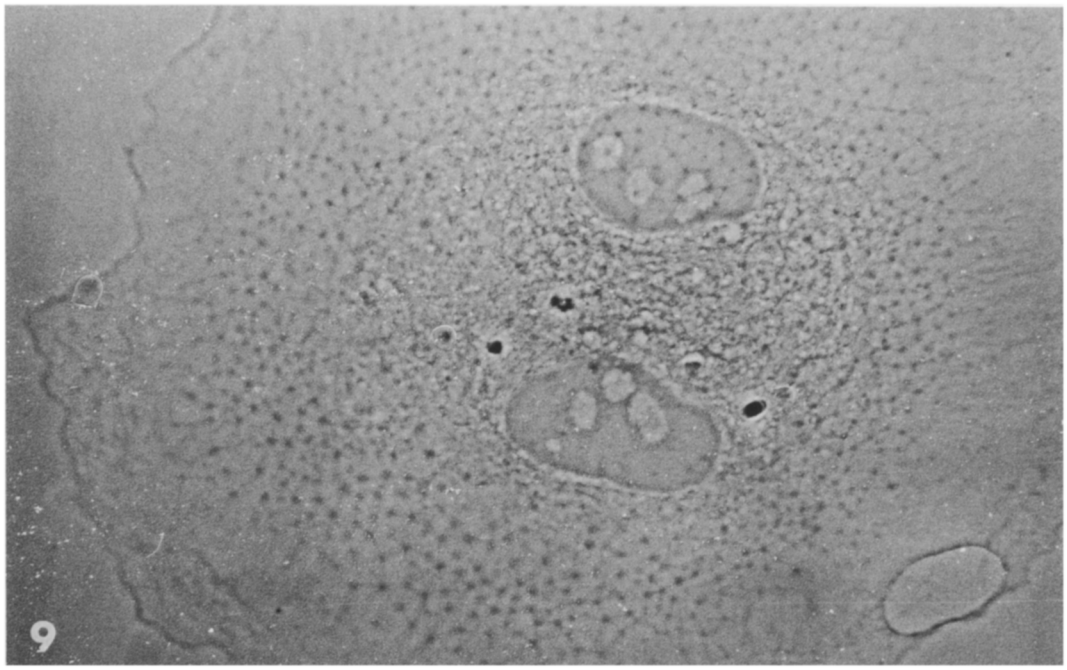
Same as Figure 4. Note the fine periodic segments as well as the fluorescent patches (Figure 6, p) revealed by the antibody. Bar = 10 μm.





Figures 7–10. Indirect Immunofluorescence with the α-Actinin Antibody on Rat Embryo Cells Approximately 8 Hr after Plating

Cells at confluency were detached from their substratum with 0.05% trypsin, 0.5 mM EDTA, and replated on glass coverslips in medium-containing serum. Approximately 8 hr after plating, the cells were fixed and processed for indirect immunofluorescence as described in Experimental Procedures. The cells were then viewed with phase contrast (Figures 7 and 9) and epifluorescent (Figures 8 and 10) optics. Bar = 10 μm.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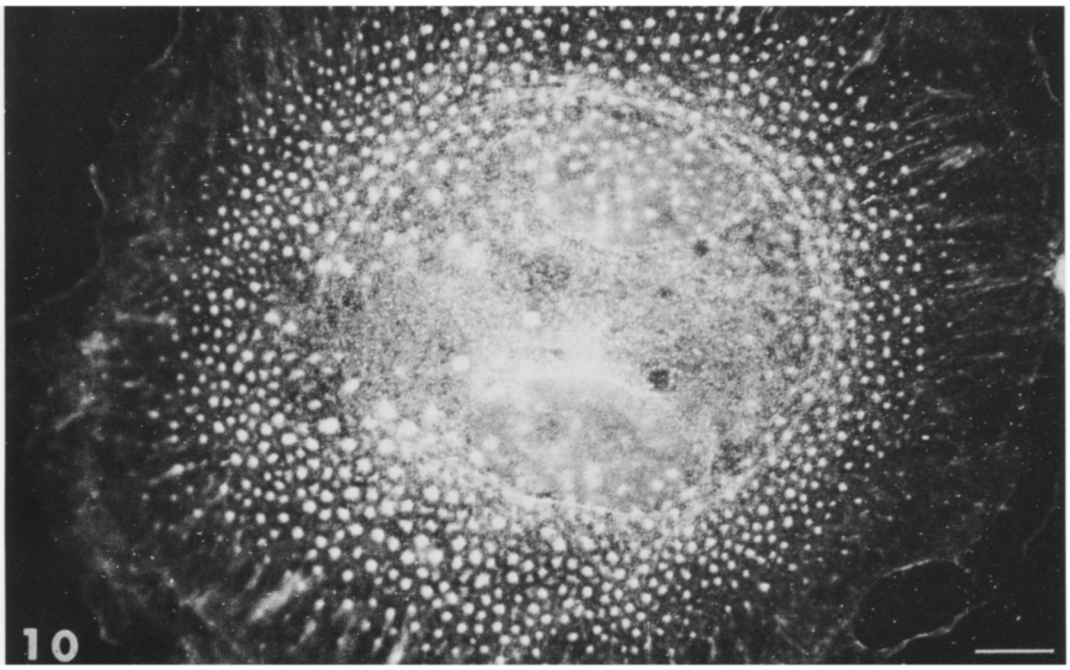
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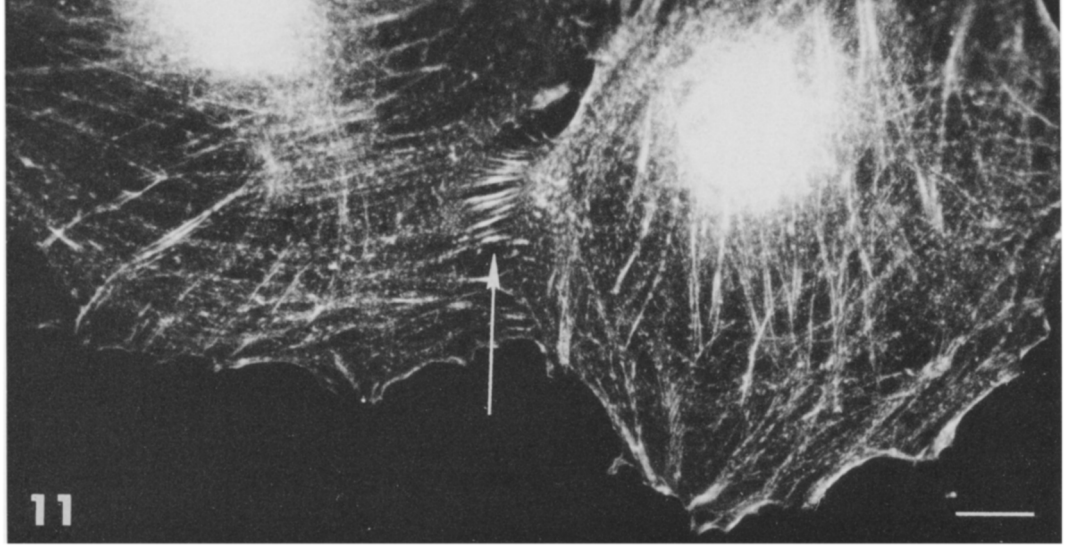

Figure 11

Same as Figures 8 and 10. The cells were fixed and processed for indirect immunofluorescence approximately 10 hr after plating. Note the presence of α-actinin in areas of cell to cell contact (arrows). Bar = 10 μm.

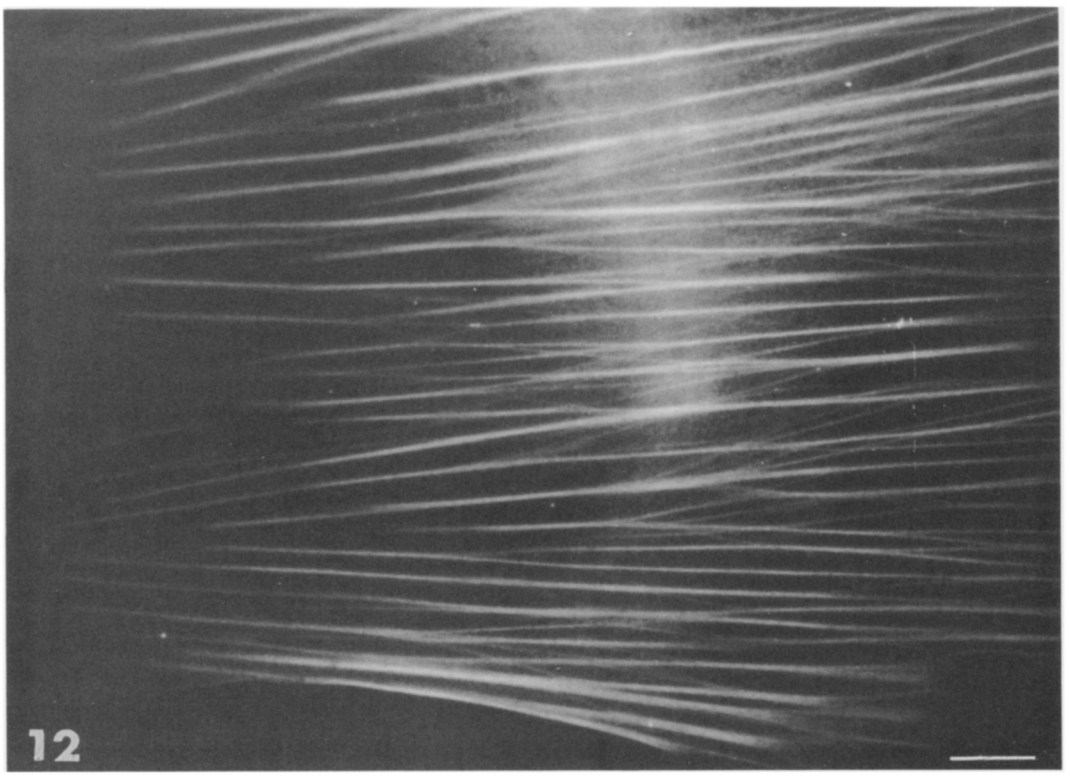

Figure 12. Indirect Immunofluorescence with the Tropomyosin and Subsequently with the α-Actinin Antibodies on a Population of Fully Spread Out Rat Embryo Cells

Cells were reacted for 1 hr with the tropomyosin antibody (Lazarides, 1975b), washed, and subsequently reacted for 1 hr with the α-actinin antibody. The sequence of application of the two antibodies did not result in any obvious difference in the staining patterns observed. At the end of the reaction of the α-actinin antibody, the cells were washed and further incubated for 1 hr with the fluorescein-labeled goat anti-rabbit IgG fraction. Bar = 10 μm.